



UM01531.ST25.txt  
SEQUENCE LISTING

<110> Hale, Calvin C  
Price, Elmer M

<120> LARGE SCALE EXPRESSION AND PURIFICATION OF RECOMBINANT PROTEINS

<130> UMO 1531.1

<140> US 09/901,419

<141> 2001-07-09

<160> 5

<170> PatentIn version 3.1

<210> 1

<211> 4087

<212> DNA

<213> Bos taurus

<220>

<221> CDS

<222> (268)..(3180)

<223>

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<221> sig\_peptide

<222> (268)..(363)

<223>

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<221> misc\_feature

<222> (3178)..()

<223> A Poly (H) affinity tag comprising 6 His residues have been inserted at the C-Terminus end of the coding region of the protein

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ggcgaacatc aactcgtgct tgaaaaatac caacttggag cccggtttga gaagctacat      180
cagagtctcg agatgcgacg ctacaatctg cagttttcac tagcttccca gtaggttggg      240
acagttggaa ctctgccatt gcccagc atg ctg cag ttc agt ctg tca ccc acc      294
                               Met Leu Gln Phe Ser Leu Ser Pro Thr
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ttg tcg atg gga ttt cac gtg ata gcc atg gtg gct ctc ttg ttt tcc      342
Leu Ser Met Gly Phe His Val Ile Ala Met Val Ala Leu Leu Phe Ser
10                               15                               20                               25

cat gtg gac cat ata agt gct gag aca gaa atg gaa gga gaa ggc aac      390
His Val Asp His Ile Ser Ala Glu Thr Glu Met Glu Gly Glu Gly Asn
                               30                               35                               40

gag act ggc gag tgt act ggc tcc tat tac tgt aag aag ggg gtg att      438
Glu Thr Gly Glu Cys Thr Gly Ser Tyr Tyr Cys Lys Lys Gly Val Ile
                               45                               50                               55

tta ccc att tgg gag ccc cag gac cct tcc ttt gga gac aaa att gct      486
Leu Pro Ile Trp Glu Pro Gln Asp Pro Ser Phe Gly Asp Lys Ile Ala
                               60                               65                               70

aga gcg act gtg tat ttt gtg gcc atg gtc tac atg ttt ctt gga gtc      534
Arg Ala Thr Val Tyr Phe Val Ala Met Val Tyr Met Phe Leu Gly Val
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tca atc att gct gac cgg ttc atg tcc tct ata gaa gtc atc acg tct      582
Ser Ile Ile Ala Asp Arg Phe Met Ser Ser Ile Glu Val Ile Thr Ser
90                               95                               100                               105

caa gag aaa gaa atc acc ata aag aaa ccc aat gga gag acc acc aag      630
Gln Glu Lys Glu Ile Thr Ile Lys Lys Pro Asn Gly Glu Thr Thr Lys
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aca act gtg agg atc tgg aat gag aca gtg tcc aac ctg acc ttg atg      678
Thr Thr Val Arg Ile Trp Asn Glu Thr Val Ser Asn Leu Thr Leu Met
                               125                               130                               135

gcc ctg ggg tct tca gct cca gag att ctc ctt tca gta atc gag gtg      726
Ala Leu Gly Ser Ser Ala Pro Glu Ile Leu Leu Ser Val Ile Glu Val
                               140                               145                               150

tgt ggc cat aac ttc act gca gga gac ctt ggc cct agc acc atc gtg      774
Cys Gly His Asn Phe Thr Ala Gly Asp Leu Gly Pro Ser Thr Ile Val
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ttt gtg aca gca gca tgg agc atc ttt gcc tat acc tgg ctt tac atc Phe Val Thr Ala Ala Trp Ser Ile Phe Ala Tyr Thr Trp Leu Tyr Ile 205 210 215	918
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caa gat gat gaa gaa gcc agg cga gaa atg gct agg att ctg aag gaa Gln Asp Asp Glu Glu Ala Arg Arg Glu Met Ala Arg Ile Leu Lys Glu 315 320 325	1254
ctc aag cag aag cat cca gag aag gaa ata gag caa tta ata gaa tta Leu Lys Gln Lys His Pro Glu Lys Glu Ile Glu Gln Leu Ile Glu Leu 330 335 340 345	1302
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caa ggg aca tat cag tgt ctg gag aac tgt ggc aca gta gcc ctg acc Gln Gly Thr Tyr Gln Cys Leu Glu Asn Cys Gly Thr Val Ala Leu Thr 410 415 420 425	1542

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gtg cat ctc agc aac gtc aaa gta tct ttg gaa gcc tcg gaa gac ggc Val His Leu Ser Asn Val Lys Val Ser Leu Glu Ala Ser Glu Asp Gly 490 495 500 505	1782
atc ctg gaa gcc agt cat gtc tct acc ctt gct tgc ctg gga tcc ccc Ile Leu Glu Ala Ser His Val Ser Thr Leu Ala Cys Leu Gly Ser Pro 510 515 520	1830
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ccc tat aag acc att gag ggg acc gcc aga ggt gga ggg gag gac ttt Pro Tyr Lys Thr Ile Glu Gly Thr Ala Arg Gly Gly Gly Glu Asp Phe 570 575 580 585	2022
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aaa tac ctg tat ggc cag cct gtc ttc agg aaa gtt cat gct aga gaa Lys Tyr Leu Tyr Gly Gln Pro Val Phe Arg Lys Val His Ala Arg Glu 650 655 660 665	2262
cat cca ctc ccc tct act ata atc acc atc gca gat gaa tat gat gac His Pro Leu Pro Ser Thr Ile Ile Thr Ile Ala Asp Glu Tyr Asp Asp 665 670 675	2310

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Cys	Gly	Glu	Glu	Lys	Leu	Pro	Ser	Cys	Phe	Asp	Tyr	Val	Met	His	Phe					
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Gln	Tyr	Ala	Asp	Ala	Ser	Ile	Gly	Asn	Val	Thr	Gly	Ser	Asn	Ala	Val					
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cac	gcg	gcc	aac	ggg	gaa	cag	ttc	aaa	gtg	tcc	cct	ggc	acg	cta	gct	2982				
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890					895					900					905					
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Arg Thr Ala Lys Leu Leu Thr Ser Cys Leu Phe Val Leu Leu Trp Leu
940 945 950

ttg tac att ttc ttc tcc tcc ctg gag gcc tac tgc cac ata aaa ggc 3174
Leu Tyr Ile Phe Phe Ser Ser Leu Glu Ala Tyr Cys His Ile Lys Gly
955 960 965

ttc taa aggaacaatc agatgtagta aatttatata tatatacata tatatatata 3230
Phe
970

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<212> PRT

<213> Bos taurus

<220>

<221> misc\_feature

<222> (3178)..()

<223> A Poly (H) affinity tag comprising 6 His residues have been inser

ted at the C-Terminus end of the coding region of the protein

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Glu Thr Glu Met Glu Gly Glu Gly Asn Glu Thr Gly Glu Cys Thr Gly
35      40      45

Ser Tyr Tyr Cys Lys Lys Gly Val Ile Leu Pro Ile Trp Glu Pro Gln
50      55      60

Asp Pro Ser Phe Gly Asp Lys Ile Ala Arg Ala Thr Val Tyr Phe Val
65      70      75      80

Ala Met Val Tyr Met Phe Leu Gly Val Ser Ile Ile Ala Asp Arg Phe
85      90      95

Met Ser Ser Ile Glu Val Ile Thr Ser Gln Glu Lys Glu Ile Thr Ile
100     105     110

Lys Lys Pro Asn Gly Glu Thr Thr Lys Thr Thr Val Arg Ile Trp Asn
115     120     125

Glu Thr Val Ser Asn Leu Thr Leu Met Ala Leu Gly Ser Ser Ala Pro
130     135     140

Glu Ile Leu Leu Ser Val Ile Glu Val Cys Gly His Asn Phe Thr Ala
145     150     155     160

Gly Asp Leu Gly Pro Ser Thr Ile Val Gly Ser Ala Ala Phe Asn Met
165     170     175

Phe Ile Ile Ile Ala Leu Cys Val Tyr Val Val Pro Asp Gly Glu Thr
180     185     190

Arg Lys Ile Lys His Leu Arg Val Phe Phe Val Thr Ala Ala Trp Ser
195     200     205

Ile Phe Ala Tyr Thr Trp Leu Tyr Ile Ile Leu Ser Val Ser Ser Pro
210     215     220

Gly Val Val Glu Val Trp Glu Gly Leu Leu Thr Phe Phe Phe Phe Pro
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260 265 270

Ile Glu His Glu Gly Asp Arg Pro Ser Ser Lys Thr Glu Ile Glu Met  
275 280 285

Asp Gly Lys Val Val Asn Ser His Val Asp Ser Phe Leu Asp Gly Ala  
290 295 300

Leu Val Leu Glu Val Asp Glu Arg Asp Gln Asp Asp Glu Glu Ala Arg  
305 310 315 320

Arg Glu Met Ala Arg Ile Leu Lys Glu Leu Lys Gln Lys His Pro Glu  
325 330 335

Lys Glu Ile Glu Gln Leu Ile Glu Leu Ala Asn Tyr Gln Val Leu Ser  
340 345 350

Gln Gln Gln Lys Ser Arg Ala Phe Tyr Arg Ile Gln Ala Thr Arg Leu  
355 360 365

Met Thr Gly Ala Gly Asn Ile Leu Lys Arg His Ala Ala Asp Gln Ala  
370 375 380

Arg Lys Ala Val Ser Met His Glu Val Asn Thr Glu Val Ala Glu Asn  
385 390 395 400

Asp Pro Val Ser Lys Ile Phe Phe Glu Gln Gly Thr Tyr Gln Cys Leu  
405 410 415

Glu Asn Cys Gly Thr Val Ala Leu Thr Ile Ile Arg Arg Gly Gly Asp  
420 425 430

Leu Thr Asn Thr Val Phe Val Asp Phe Arg Thr Glu Asp Gly Thr Ala  
435 440 445

Asn Ala Gly Ser Asp Tyr Glu Phe Thr Glu Gly Thr Val Val Phe Lys  
450 455 460

Pro Gly Glu Thr Gln Lys Glu Ile Arg Val Gly Ile Ile Asp Asp Asp  
465 470 475 480

Ile Phe Glu Glu Asp Glu Asn Phe Leu Val His Leu Ser Asn Val Lys  
485 490 495



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Val Ser Leu Glu Ala Ser Glu Asp Gly Ile Leu Glu Ala Ser His Val  
 500 505 510  
 Ser Thr Leu Ala Cys Leu Gly Ser Pro Ser Thr Ala Thr Val Thr Ile  
 515 520 525  
 Phe Asp Asp Asp His Ala Gly Ile Phe Thr Phe Glu Glu Pro Val Thr  
 530 535 540  
 His Val Ser Glu Ser Ile Gly Ile Met Glu Val Lys Val Leu Arg Thr  
 545 550 555 560  
 Ser Gly Ala Arg Gly Asn Val Ile Val Pro Tyr Lys Thr Ile Glu Gly  
 565 570 575  
 Thr Ala Arg Gly Gly Gly Glu Asp Phe Glu Asp Thr Cys Gly Glu Leu  
 580 585 590  
 Glu Phe Gln Asn Asp Glu Ile Val Lys Thr Ile Ser Val Lys Val Ile  
 595 600 605  
 Asp Asp Glu Glu Tyr Glu Lys Asn Lys Thr Phe Phe Leu Glu Ile Gly  
 610 615 620  
 Glu Pro Arg Leu Val Glu Met Ser Glu Lys Lys Ala Leu Leu Leu Asn  
 625 630 635 640  
 Glu Leu Gly Gly Phe Thr Ile Thr Gly Lys Tyr Leu Tyr Gly Gln Pro  
 645 650 655  
 Val Phe Arg Lys Val His Ala Arg Glu His Pro Leu Pro Ser Thr Ile  
 660 665 670  
 Ile Thr Ile Ala Asp Glu Tyr Asp Asp Lys Gln Pro Leu Thr Ser Lys  
 675 680 685  
 Glu Glu Glu Glu Arg Arg Ile Ala Glu Met Gly Arg Pro Ile Leu Gly  
 690 695 700  
 Glu His Thr Arg Leu Glu Val Ile Ile Glu Glu Ser Tyr Glu Phe Lys  
 705 710 715 720  
 Ser Thr Val Asp Lys Leu Ile Lys Lys Thr Asn Leu Ala Leu Val Val  
 725 730 735  
 Gly Thr Asn Ser Trp Arg Glu Gln Phe Ile Glu Ala Ile Thr Val Ser

740

745

750

Ala Gly Glu Asp Asp Asp Asp Asp Glu Cys Gly Glu Glu Lys Leu Pro  
 755 760 765

Ser Cys Phe Asp Tyr Val Met His Phe Leu Thr Val Phe Trp Lys Val  
 770 775 780

Leu Phe Ala Phe Val Pro Pro Thr Glu Tyr Trp Asn Gly Trp Ala Cys  
 785 790 795 800

Phe Ile Val Ser Ile Leu Met Ile Gly Leu Leu Thr Ala Phe Ile Gly  
 805 810 815

Asp Leu Ala Ser His Phe Ala Cys Thr Ile Ala Leu Lys Asp Ser Val  
 820 825 830

Thr Ala Val Val Phe Val Ala Leu Gly Thr Ser Val Pro Asp Thr Phe  
 835 840 845

Ala Ser Lys Val Ala Ala Thr Gln Asp Gln Tyr Ala Asp Ala Ser Ile  
 850 855 860

Gly Asn Val Thr Gly Ser Asn Ala Val Asn Val Phe Leu Gly Ile Gly  
 865 870 875 880

Val Ala Trp Ser Ile Ala Ala Ile Tyr His Ala Ala Asn Gly Glu Gln  
 885 890 895

Phe Lys Val Ser Pro Gly Thr Leu Ala Phe Ser Val Thr Leu Phe Thr  
 900 905 910

Ile Phe Ala Phe Ile Asn Val Gly Val Leu Leu Tyr Arg Arg Arg Pro  
 915 920 925

Glu Ile Gly Gly Glu Leu Gly Gly Pro Arg Thr Ala Lys Leu Leu Thr  
 930 935 940

Ser Cys Leu Phe Val Leu Leu Trp Leu Leu Tyr Ile Phe Phe Ser Ser  
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Leu Glu Ala Tyr Cys His Ile Lys Gly Phe  
 965 970

&lt;210&gt; 3

&lt;211&gt; 970

&lt;212&gt; PRT

&lt;213&gt; Bos taurus

&lt;400&gt; 3

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Ile Ala Met Val Ala Leu Leu Phe Ser His Val Asp His Ile Ser Ala  
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Glu Thr Glu Met Glu Gly Glu Gly Asn Glu Thr Gly Glu Cys Thr Gly  
 35 40 45

Ser Tyr Tyr Cys Lys Lys Gly Val Ile Leu Pro Ile Trp Glu Pro Gln  
 50 55 60

Asp Pro Ser Phe Gly Asp Lys Ile Ala Arg Ala Thr Val Tyr Phe Val  
 65 70 75 80

Ala Met Val Tyr Met Phe Leu Gly Val Ser Ile Ile Ala Asp Arg Phe  
 85 90 95

Met Ser Ser Ile Glu Val Ile Thr Ser Gln Glu Lys Glu Ile Thr Ile  
 100 105 110

Lys Lys Pro Asn Gly Glu Thr Thr Lys Thr Thr Val Arg Ile Trp Asn  
 115 120 125

Glu Thr Val Ser Asn Leu Thr Leu Met Ala Leu Gly Ser Ser Ala Pro  
 130 135 140

Glu Ile Leu Leu Ser Val Ile Glu Val Cys Gly His Asn Phe Thr Ala  
 145 150 155 160

Gly Asp Leu Gly Pro Ser Thr Ile Val Gly Ser Ala Ala Phe Asn Met  
 165 170 175

Phe Ile Ile Ile Ala Leu Cys Val Tyr Val Val Pro Asp Gly Glu Thr  
 180 185 190

Arg Lys Ile Lys His Leu Arg Val Phe Phe Val Thr Ala Ala Trp Ser  
 195 200 205

Ile Phe Ala Tyr Thr Trp Leu Tyr Ile Ile Leu Ser Val Ser Ser Pro  
 210 215 220

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Gly Val Val Glu Val Trp Glu Gly Leu Leu Thr Phe Phe Phe Phe Pro  
 225 230 235 240  
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 245 250 255  
 Lys Tyr Val Tyr Lys Arg Tyr Arg Ala Gly Lys Gln Arg Gly Met Ile  
 260 265 270  
 Ile Glu His Glu Gly Asp Arg Pro Ser Ser Lys Thr Glu Ile Glu Met  
 275 280 285  
 Asp Gly Lys Val Val Asn Ser His Val Asp Ser Phe Leu Asp Gly Ala  
 290 295 300  
 Leu Val Leu Glu Val Asp Glu Arg Asp Gln Asp Asp Glu Glu Ala Arg  
 305 310 315 320  
 Arg Glu Met Ala Arg Ile Leu Lys Glu Leu Lys Gln Lys His Pro Glu  
 325 330 335  
 Lys Glu Ile Glu Gln Leu Ile Glu Leu Ala Asn Tyr Gln Val Leu Ser  
 340 345 350  
 Gln Gln Gln Lys Ser Arg Ala Phe Tyr Arg Ile Gln Ala Thr Arg Leu  
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 Met Thr Gly Ala Gly Asn Ile Leu Lys Arg His Ala Ala Asp Gln Ala  
 370 375 380  
 Arg Lys Ala Val Ser Met His Glu Val Asn Thr Glu Val Ala Glu Asn  
 385 390 395 400  
 Asp Pro Val Ser Lys Ile Phe Phe Glu Gln Gly Thr Tyr Gln Cys Leu  
 405 410 415  
 Glu Asn Cys Gly Thr Val Ala Leu Thr Ile Ile Arg Arg Gly Gly Asp  
 420 425 430  
 Leu Thr Asn Thr Val Phe Val Asp Phe Arg Thr Glu Asp Gly Thr Ala  
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His Val Ser Glu Ser Ile Gly Ile Met Glu Val Lys Val Leu Arg Thr  
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Ser Gly Ala Arg Gly Asn Val Ile Val Pro Tyr Lys Thr Ile Glu Gly  
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Thr Ala Arg Gly Gly Gly Glu Asp Phe Glu Asp Thr Cys Gly Glu Leu  
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Glu Phe Gln Asn Asp Glu Ile Val Lys Thr Ile Ser Val Lys Val Ile  
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Asp Asp Glu Glu Tyr Glu Lys Asn Lys Thr Phe Phe Leu Glu Ile Gly  
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Glu Pro Arg Leu Val Glu Met Ser Glu Lys Lys Ala Leu Leu Leu Asn  
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Glu Leu Gly Gly Phe Thr Ile Thr Gly Lys Tyr Leu Tyr Gly Gln Pro  
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Ile Thr Ile Ala Asp Glu Tyr Asp Asp Lys Gln Pro Leu Thr Ser Lys  
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Glu Glu Glu Glu Arg Arg Ile Ala Glu Met Gly Arg Pro Ile Leu Gly  
690 695 700

Glu His Thr Arg Leu Glu Val Ile Ile Glu Glu Ser Tyr Glu Phe Lys  
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Ser Thr Val Asp Lys Leu Ile Lys Lys Thr Asn Leu Ala Leu Val Val  
Page 13

725

730

735

Gly Thr Asn Ser Trp Arg Glu Gln Phe Ile Glu Ala Ile Thr Val Ser  
 740 745 750

Ala Gly Glu Asp Asp Asp Asp Asp Glu Cys Gly Glu Glu Lys Leu Pro  
 755 760 765

Ser Cys Phe Asp Tyr Val Met His Phe Leu Thr Val Phe Trp Lys Val  
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Leu Phe Ala Phe Val Pro Pro Thr Glu Tyr Trp Asn Gly Trp Ala Cys  
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Phe Ile Val Ser Ile Leu Met Ile Gly Leu Leu Thr Ala Phe Ile Gly  
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Thr Ala Val Val Phe Val Ala Leu Gly Thr Ser Val Pro Asp Thr Phe  
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Ala Ser Lys Val Ala Ala Thr Gln Asp Gln Tyr Ala Asp Ala Ser Ile  
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Gly Asn Val Thr Gly Ser Asn Ala Val Asn Val Phe Leu Gly Ile Gly  
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Val Ala Trp Ser Ile Ala Ala Ile Tyr His Ala Ala Asn Gly Glu Gln  
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Phe Lys Val Ser Pro Gly Thr Leu Ala Phe Ser Val Thr Leu Phe Thr  
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Ile Phe Ala Phe Ile Asn Val Gly Val Leu Leu Tyr Arg Arg Arg Pro  
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